Alu Elements: Repetitive DNA as Facilitators of Chromosomal Rearrangement

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Abstract

Alu repeats are the most common type of repetitive DNA sequences dispersed throughout the human genome. Technical advances in the field of cytogenetics and molecular biology have facilitated the analysis of epithelial tumors and hematologic malignancies which has led to the observation of *Alu* elements in and near sites often involved in chromosomal rearrangements. Repair mechanisms of double strand breaks (DSB) such as homologous recombination (HR) may rely on the sequence homology of *Alu* repeats, potentially leading to chromosomal rearrangements. Databases have confirmed the strong association between *Alu* repeats, specifically the 26 bp consensus sequence and chromosomal regions involved in deletions and translocations. Although the *Alu* repetitive sequence is a potential "hotspot" during homologous recombination, there are other cellular mechanisms that may play a more prominent role in the initiation of chromosomal rearrangements.

Introduction

Chromosomal rearrangements are hallmarks of tumor cells, and technical improvements in cytogenetic and molecular biology techniques in recent decades have led to the identification of many recurrent translocations, deletions and inversions that are characteristic of a variety of hematologic and solid tumor malignancies. Many different events can initiate chromosomal rearrangements, including spontaneous chromosome breakage, unequal crossing over, exposure to certain chemicals and viruses. Nevertheless, despite extensive study, the mechanisms that generate these events are not as yet completely understood.

In recent years, investigators have demonstrated the involvement of *Alu* repeat mediated recombination in the creation of chromosomal aberrations (see review by Kolomietz et al., 2002). The present article summarizes the basic findings of such research, focusing on the role of *Alu* repeats in the genesis of chromosomal aberrations observed in malignant cells, both from epithelial tumors and hematological malignancies.

Historical Background of the Study of Tumor Cells

The study of tumor cells was first published in 1890 by David von Hansemann who discovered mitotic abnormalities in malignant tissue. In 1914, Theodore Boveri published his somatic mutation theory that genetic imbalances of the cell's mitotic structures could lead to chromosomal aneuploidy, the initiating factor in tumorigenesis. However, at that time, the precise mechanisms that contributed to the phenotype of a cancer cell could not be verified due to technical limitations in visualizing chromosomes. The discovery of the correct number of human chromosomes by Tjio and Levan in 1956 was followed by the landmark finding of the Philadelphia chromosome and its association with chronic myelogenous leukemia (CML) in 1960 by Nowell and Hungerford. The 1970s ushered in advances in chromosomal banding techniques which allowed for much more accurate chromosomal identification, leading to the analysis of karyotypic changes that characterize leukemias and lymphomas.

Since the 1980s, the integration of molecular biology and cytogenetics has tremendously broadened the analysis of rearrangements and other structural aberrations that result from chromosomal exchanges. Fluorescence *in situ* hybridization (FISH) has been invaluable for detecting chromosomal translocations, identifying the chromosomal composition of marker chromosomes, and revealing the presence of subtle, cryptic chromosomal abnormalities that would otherwise go undetected by conventional banding methodologies (Gall et al., 1969; John et al., 1969; Montgomery et al.,1997). Spectral karyotyping (SKY), a cytogenetic technique based on FISH that allows for the visualization of all chromosomes at one time with each chromosome identified by a unique combination of fluorescent dyes, has been of enormous benefit in the elucidation of complex chromosomal rearrangements, both in humans and in rodents (Schröck et al., 1996; Liyanage et al., 1996). Comparative genomic hybridization (CGH) is another molecular cytogenetic technique which has proven useful in the study of tumor cells. It involves the competitive hybridization reaction between differentially labeled DNA from normal cells and tumor DNA on normal metaphase chromosomes, thereby identifying tumor specific genome-wide patterns of chromosomal gains and losses in tumor samples (Kallioniemi et al., 1992). This technique eliminates the challenge of preparing chromosomes from tumor samples which are often short, fuzzy and have a low mitotic index. With CGH, DNA can be isolated from either fresh or archived tumors, eliminating the arduous task of chromosome preparation from tumor cells.

Sequence Dependent Regions Involved in Recurrent Translocations

Recent advances in the fields of cytogenetics and molecular biology have introduced a greater understanding of the molecular mechanisms that are involved in the formation of recurrent translocations in cancers. Certain specific regions within chromosomes have been identified as being relevant in tumorigenesis and are thought to make DNA more susceptible to recombination. One type of these regions, known as repetitive DNA, contains sequences that are present in more than one copy. These repetitive sequences account for more than a third of the human genome and are ubiquitously interspersed throughout the genome. Recombination occasionally occurs between the interspersed repeats and can interrupt sequences, consequently altering gene function (Schmid, 1996).

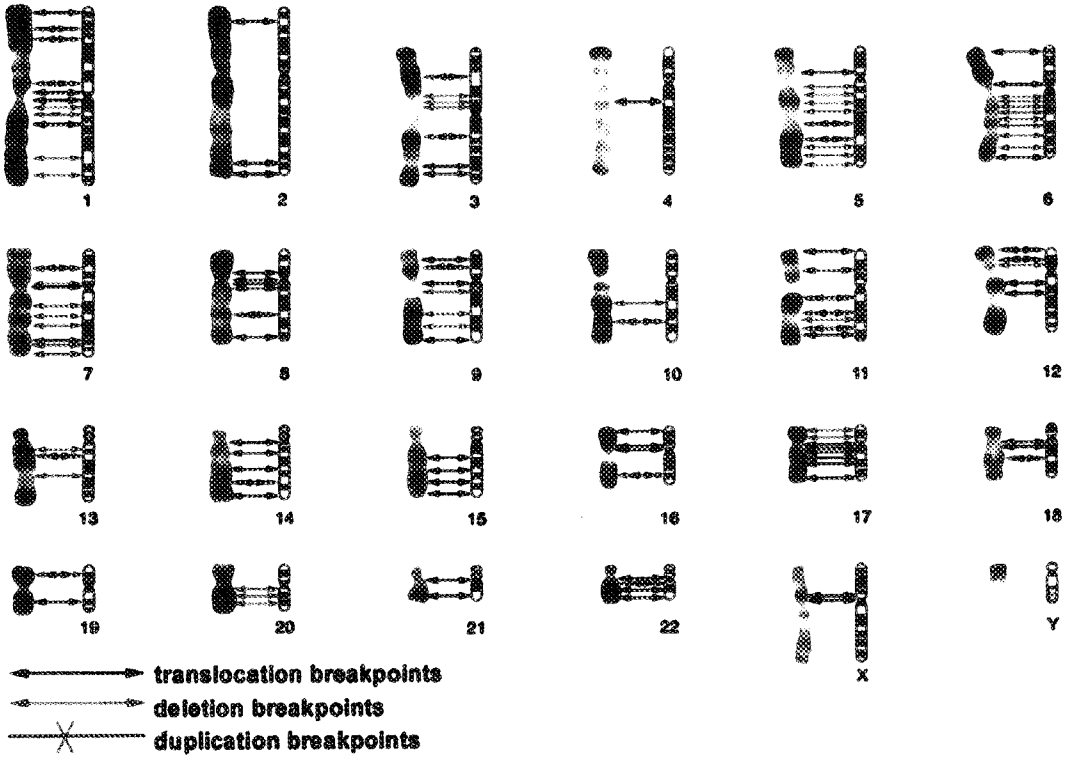

Figure 1: Breakpoints of recurrent chromosomal aberrations observed in cancer that correspond to Alu rich sites within R-bands (Figure from Kolomietz et al. The Role of Alu Repeat Clusters as Mediators of Recurrent Chromosomal Aberrations in Tumors. Genes Chromosomes and Cancer. 2002; 35:97-112. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.)

Alu Elements

Two major classes of repetitive sequences are LINES (long interspersed elements) and SINES (short interspersed elements). *Alu* sequences are the most prevalent type of SINE, comprising about 500,000 to one million copies of repeats, and account for 5% to 10% of the human genome. Given their frequency, *Alu* elements have been implicated in a variety of mechanisms involving genomic rearrangement, the regulation of gene expression, imprinting, recombination, meiotic mutations, etc. (Kolomietz et al., 2002).

Alu elements are 282 base pairs (bp) long, consisting of a 26 bp core with a defined 5' end (that is observed in most of its members) and a divergent tandem dimer. There are several subfamilies of *Alu* elements and individual *Alu* family members are highly homologous to each other. Certain regions of the genome are much more densely populated with *Alu* repeats and it has been shown that they are preferentially localized to the metaphase chromosome areas known as reverse bands (R-bands) (Holmquist, 1992; Craig and Bickmore, 1993). Most of the mapped human genes can be found in R bands (Tamayo, 2003). Figure 1 shows the position of known breakpoint regions frequently involved in genomic rearrangements in cancer. These regions also correspond to the position of *Alu* repeats on R-banded human chromosomes (Kolomietz et al., 2002).

Some recent studies have suggested that the 26 bp *Alu* core itself can promote genomic rearrangement. In one such study, Rudiger et al. (1995) analyzed the rearrangements in the LDL (low density lipoprotein)-receptor gene of patients with familial hypercholesterolemia. The LDL-receptor gene is unique because although *Alu* elements are present in the 17 introns of the gene, they can also be found in the untranslated part of the last exon. After examining similarities and differences of sequences involved in recombinational events, it was discovered that the 26 bp consensus sequence of the *Alu* element was located either upstream or downstream in all of the LDL-receptor gene sequences (Figure 2). Thus, it is possible that recombination may be due to a preference for this sequence.

Homologous Recombination and *Alu* Elements

Accurate repair of damage to DNA is crucial to maintain the integrity of the genome and to prevent chromosomal rearrangements. Homologous recombination (HR) is one of the two main pathways for the repair of double-strand breaks (DSBs) in mammalian cells and it functions during the late S-G_2 phase of the cell cycle. DSBs can be generated by DNA-damaging agents such as ionizing radiation and oxygen radicals, by RAG proteins in V(D)J recombination, and during replication (Szostak et al.,1983; Bishop et al., 2000; Lee et al., 2004). When DNA damage induces HR (HR seeks sequence homology that is similar to a region in the damaged strand) an endonuclease cleaves DNA, leaving free 3' and 5' ends on each strand so that the damaged area can be removed. The template may be from a homologous chromosome, an undamaged sister chromatid, or sequence repeats on non-homologous chromosomes (i.e., *Alu* elements). Using this sequence homology as a template, DNA polymerase fills in the strand with the correct nucleotides. DNA ligase then seals the free ends.

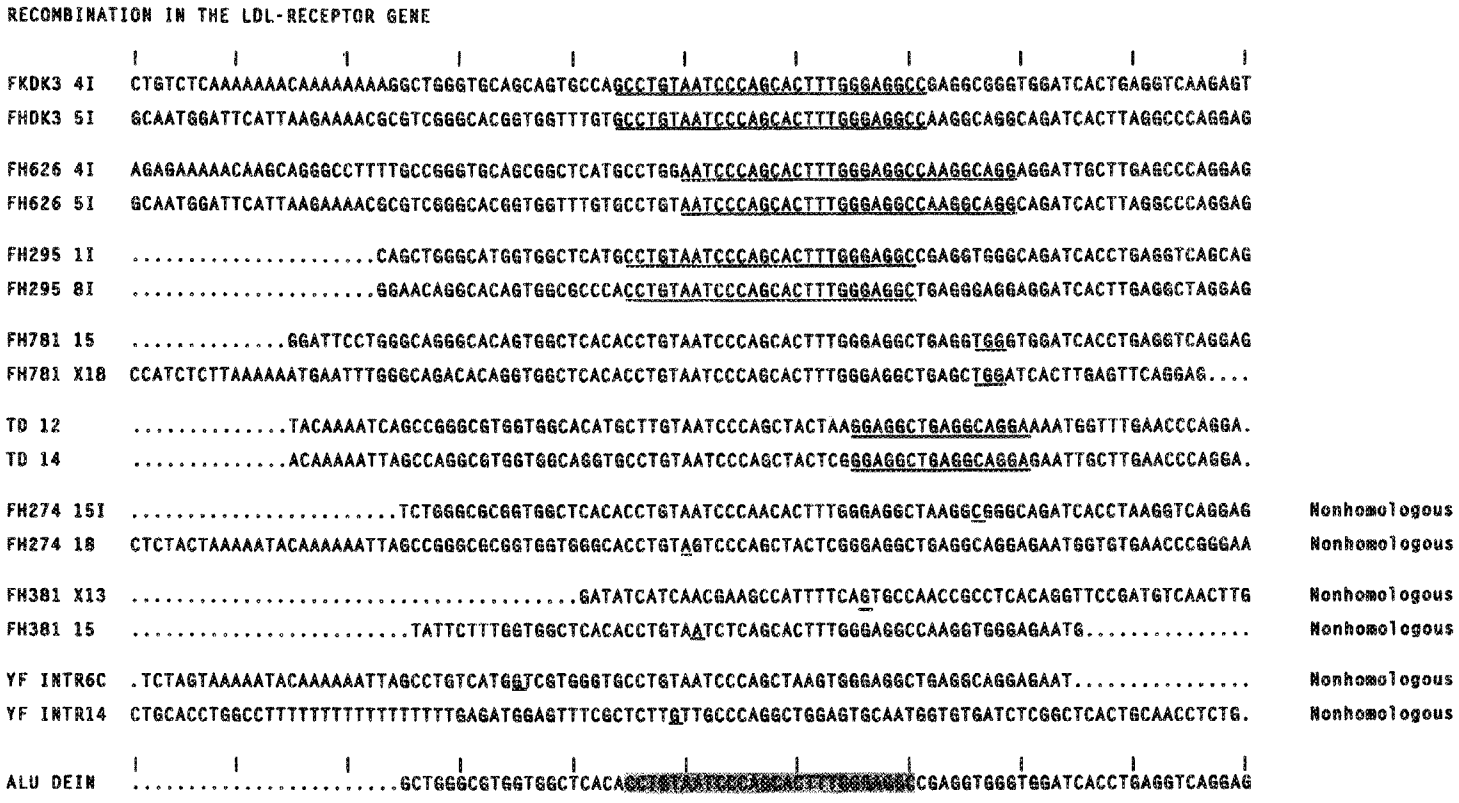

Figure 2. Recombination of the LDL-receptor gene involving Alu repeats. In a study of patients with familial hypercholesterolemia, the LDL-receptor gene was observed in eight rearrangements that could be classified as homologous and non-homologous recombination. Five of these recombination events show sequence homology to the *Alu* repetitive sequence reported by Deininger et al. (1981). Three are classified as nonhomologous (indicated). The prefix before the strands denotes the names of the patients in whom the sequences have been found. The number following the prefix corresponds to the intron number of the parental sequence except where 'X' (exon) is added. The 'I' at the end indicates the orientation opposite of transcription and the lower strand is presented. The regions where the bases are underlined denote the sites of recombination. Therefore, because the 26bp core is highly conserved in these *Alu* elements, this sequence may be considered a recombinational hotspot. (Figure modified from Rudiger et al. One short well conserved region of Alu-sequences is involved in human gene rearrangements and has homology with prokaryotic chi. Nucleic Acids Res. 1995; 23(2):256-260. Reprinted with permission of Oxford University Press.)

Alu Elements and Chromosomal Rearrangements

The repetitive sequences of *Alu* elements can serve as sites for unequal crossing over. The *Alu* sequences may base pair following double strand breaks or by physical juxtaposition, or they may act as substrates for homologous recombination. Homologous recombination (HR) is mediated by similar regions of homologous chromosomes, including interspersed repetitive elements such as *Alu* sequences. Unequal recombination of such sequences often yield deletions and duplications and translocations when nonhomologous chromosomes are involved (Abeysinghe et al., 2003). Deininger and Batzer (1999) also have shown that *Alu* mediated recombination occurring inter-chromosomally (in trans) results in complex chromosomal translocations while unequal crossing over between *Alu* elements occurring intra-chromosomally (in cis) results in deletions or duplications of intervening sequences.

Repair mechanisms such as HR between *Alu* elements and other homologous genomic sequences may result in recombination (Morris et al., 1996). Conversely, Neves et al. (1999) asserts that the chance of rearrangement is increased due to the attraction between *Alu* elements on nonhomologous chromosomes.

Others have observed that when *Alu* elements are present on recombinant DNA constructs, they show increased recombination frequency between vector DNA and host genomic loci (Kato et al., 1986; Wallenburg et al., 1987; Kang et al., 1999). This evidence supports the theory that *Alu* elements are potential "hot spots" for recombination events and mediate chromosomal translocations.

Alu Repeats Responsible for Human Disease

A recent study was conducted to pinpoint the sequences involved in translocations and gross deletion breakpoints that are

Table 1. Human diseases associated with *Alu* repeats.
(Table adapted from Kolomietz et al., 2002; Deininger and Batzer 1999)

Disease	Locus or Gene	Reference
Acute myeloid and lymphoid leukemia	MLL dup(11)(q23)	Strout et al., 1998; Wiedemann et al., 1999
Treatment-related acute lymphoblastic leukemia	t(4;11)(q21;q23)	Megonigal et al., 1997
De novo acute myeloid leukemia	t(9;11)(p22;q23)	Super et al., 1997
CLL	t(14;19)(q32;q13)	Ohno et al., 1993
CML, Ph positive acute leukemia	Ph translocation t(9;22)(q34;q11)	Martinelli et al., 2000
CML	Variant Ph translocation	Jeffs et al., 1998
Burkitt lymphoma cell line	t(2;8)(p11;q24)	Kato et al., 1991
Follicular lymphomas (FLs)	t(14;18)(q32;q21)	Buchonnet et al., 2000
Fanconi's anemia	*FANCA* (16q24.3)	Morgan et al., 1999
Breast and ovarian cancer	*BRCA1 (17q21)*	Swensen et al., 1997; Montagna et al., 1999; Rohlfs et al., 2000
Ewing Sarcoma	t(11;22)(q24;q12)	Obata et al., 1999
Subset of Ewing sarcoma	*EWSR1* (22q12.2)	Zucman-Rossi et al., 1997
Association with glioma	*RB1* (13q14.2)	Rothberg et al., 1997
Familial colorectal cancer	*MLH1*(3p21.3)	Mauillon et al., 1996
Hereditary non polyposis colorectal cancer	*MSH2* human DNA mismatch gene (2p22-p21)	Marshall et al., 1996
Duchenne muscular dystrophy	Dystrophin (Xq22)	Hu et al., 1991
Ehlers-Danlos syndrome	Lysine hydroxylase (2q31)	Pousi et al., 1994
Fabry disease	Alpha--galactosidase A (Xq22)	Kornreich et al., 1990
Lesch-Nyhan	*HPRT* (Xq26.1)	Tvrdik et al., 1998; Marcus et al., 1993
Neurofibromatosis type 1	*NF1 (17q11.2)*	Wallace et al., 1991; Xu et al., 1991
Tay-Sachs disease	β-Hexosaminidase α-chain gene (15q23-q24)	Myerowitz and Hogikyan, 1987
α-Thalessemia	α-globin gene cluster (16p13.3)	Harteveld et al., 1997

associated with human inherited disease and cancer. In establishing the Gross Rearrangement Breakpoint Database (GRaBD) (www.uwcm.ac.uk/uwcm/mg/grabd/grabd.html), Abeysinghe et al. (2003) analyzed 397 chromosomal rearrangement breakpoint junctions. They screened for the presence of repetitive elements using the Repbase database (www.girinst.org) and the RepeatMasker Program (http://woody.embl-heidelberg.de/repeatmask). These programs found 102 repetitive sequences, 80 of which were located at breakpoint junctions. *Alu* elements were determined to be the most abundant sequence found at the breakpoint junctions of deletions and translocations analyzed by the GraBD.

Many different constitutional diseases and some germline diseases have been associated with unequal homologous recombination between *Alu* repeats (Huie et al., 1999). After identifying sixteen cases of cancer attributed to the insertion of *Alu* elements, Deininger and Batzer (1999) suggest that 0.1% of human genetic diseases could be generated by *Alu* insertion alone. Table 1 is a summary of some human diseases with translocations or deletions that are attributed to the presence of *Alu* repeats. While some of these diseases have *Alu* repeats within the breakpoint regions, in others, *Alu* repeats are in close proximity to the breakpoints. Likewise, *Alu* repeats are also located near a partial duplication of the *MLL* gene in AML (acute myeloid leukemia) (Kolomietz et al., 2002).

Deletions Involving *Alu* Repeats

Salagnick and Dianov (1992) observed that deletions resulted from the base-pairing of direct repeats flanking the DNA broken ends of DSBs. When the flanking sequences of broken ends join together, the intervening sequences are deleted. Small deletions were observed, ranging from several to 1,500 nucleotides.

A number of human cancers result from small deletions within certain chromosomal regions. In a study of colorectal cancer, Plaschke et al. (2003) found a deletion in the promoter region of the *hMSH6* (mutS homolog 6) gene that likely was mediated by recombination between homologs of the Sx family of *Alu* repeats. Rohlfs et al. (2000) identified a deletion of the *BRCA1* (breast cancer 1) gene in breast cancer families resulting from recombination between closely related *Alu* repeats. The *RB1* (retinoblastoma) tumor suppressor gene was observed to be deleted in a small population of brain cancer patients. This deletion was presumed to be caused by homologous recombination between two *Alu* repeats (Rothberg et al., 1997).

Kolomietz et al. (2002) reported that deletions have been found immediately adjacent to breakpoint regions in about 10% of leukemia-associated chromosomal rearrangements. They found deletions adjacent to the two oncogenes, *ABL* and *BCR*, which are rearranged in the formation of the Philadelphia chromosome, in almost 10% of patients with CML and Ph+ ALL. To examine the relationship of deletion sequences and their association to chromosomal rearrangement, they submitted the DNA sequences of the genes involved in the chromosomal rearrangements and their flanking regions to repeat identification programs such as Censor (http://www.girinst.org/Censor_ServerData_Entry_Forms.html) and Repeat Masker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker). The results showed a strong association between the propensity to undergo deletion and a high density of *Alu* repeats in the chromosomal regions involved in rearrangement. Sinclair et al. (2000) discovered large recurrent deletions at the t(9;22) breakpoint junction which they thought may identify a poor prognosis subgroup of patients with CML. It has been demonstrated that deletions of base pairs can occur due to exonuclease activity on the broken ends following double strand breakage (Szostak et al., 1983; Zucman-Rossi et al., 1998). Each of these studies show that the deletions are associated with different chromosomal rearrangements, thereby suggesting that there may be a common mechanism for deletion formation that is sequence specific rather than disease specific.

Despite the fact that chromosomal deletions occur, *Alu* mediated deletion is still low, with rates of less than $7x10^{-7}$ and a maximum frequency of somatic mutation of less than 10^{-6} per cell (Hollies et al., 2001).

Discussion

The high density of *Alu* elements in the human genome and evidence that sequence dependent homologous recombination is a major DNA repair pathway indicates that there must be a mechanism capable of regulating unequal homologous recombination among dispersed *Alu* elements and preventing chromosomal instability.

Deininger and Batzer (1999) point out that there is evidence that recombination at *Alu* elements may be more complex than simple homologous recombination. In the study by Rudiger et al. (1995) the LDL-receptor gene was observed in recombination events that involved the specific location of the 26 bp core sequence within the *Alu* element. They and other groups have demonstrated that this core sequence stimulates recombination and could be a hotspot for a mechanism more broad than homologous recombination.

However, in several genetic diseases such as ataxia-telangiectasia (AT) that have a DNA instability phenotype and a high frequency of carcinogenesis, some genes function as part of a signaling network in the repair of DSB. Typically the *ATM* gene (defective in AT) is a regulator in a cellular checkpoint mechanism that repairs DSB and maintains cellular survival (Thompson and Schild, 2002). Since it has been demonstrated that repetitive elements can serve as sites for unequal homologous crossing over, potentially leading to translocations and deletions (Kolomietz et al. 2002), the example of *ATM* shows that the recombinogenic effects of *Alu* elements based on sequence homology may be regulated by more than just overlap of other repair mechanisms (i.e., nonhomologous endjoining and single-strand annealing) in addition to such as HR.

Other factors, such as other repair mechanisms, cell cycle control, or replication are involved in the repair of double strand breakage and in the maintenance of the stability of the genome. However, because there are so many different regulatory mechanisms and different types of DSBs, at the present time no one particular regulating mechanism has been implicated.

Recent advances in the fields of cytogenetics and molecular biology have produced a greater understanding of the molecular mechanisms that are involved in the formation of recurrent translocations occurring in cancers. While some researchers have begun looking at oligonucleotide sequences involved in translocation breakpoints (Abeysinghe et al. 2003), future directions towards understanding the role of *Alu* repeats in chromosomal rearrangement may include the analysis of the complete human genome sequence for comparisons of the presence of *Alu* repeats and cancer breakpoints.

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Glossary

Alu elements – the most common class of repetitive sequences that are ubiquitously interspersed throughout the genome; ~300 base pairs (bp) long; the name is derived from the restriction site Alu I; also called *Alu* repeats and *Alu* sequences.

SINES – short interspersed elements (100-400 bp long), a class of repetitive nucleic acid sequences, including *Alu* elements, that are widely dispersed throughout the human genome; derived from transcripts of RNA polymerase III.

LINES – long interspersed elements (~6,500 bp long), a class of repetitive nucleic acid sequences that are widely dispersed throughout the human genome; derived from RNA polymerase II transcripts.

Double Strand Break (DSB) – a type of lesion occurring in both strands of DNA that separates the two strands resulting in two fragments as opposed to single strand breaks.

Homologous Recombination (HR) – a mechanism of recombination in mammalian cells that utilizes homologous sequences of DNA for the repair of double strand breaks.

Endonucleases – enzymes that cleave bonds at specific short sequences within DNA or RNA, creating internal breaks; double stranded and single stranded nucleic acids may be cleaved.

Exonucleases – enzymes that digest nucleotides one at a time from the end of a polynucleotide chain; they can function at either the 5' or 3' end of DNA or RNA.